Original article

Pertussis seroimmunity in mother-neonate pairs and other pediatric age groups from Egypt

Background: *Despite the widespread availability of 2 classes of effective vaccines, whole cell and acellular, pertussis has resurged as a serious public health problem. We sought to investigate the pertussis immune status of mother-neonate pairs and children in our country where pertussis vaccination is obligatory.* ***Methods:*** *This cross-sectional study included 75 healthy full-term neonates and their mothers, 100 infants (2-24 months), 170 children (2-12 years) and 80 adolescents (12-18 years). Serum pertussis IgG was measured in all enrolled subjects. A positive titre was defined as >24 U/ml.* ***Results:*** *Positive pertussis IgG levels were detected in 69 of the mothers (92%), in 63 of their newborns (84%). Seroimmunity to pertussis was positively noted in 55% of infants, 82.2% of preschool children, 77.5% of school-aged children and 75% in adolescents. Serum pertussis IgG titers among the neonates showed a significant positive correlation with the maternal titers (P=0.00001). Higher rates of pertussis seroimmunity was observed among residents in urban and suburban areas as compared to those living in rural areas (P<0.05).* ***Conclusion:*** *This pilot study may suggest the presence of sufficient pertussis seroimmunity rates in the studied age groups. Still, there were some failures in immune acquisition probably due to inefficient vaccination in some localities or waning of immunity with age. Wider scale studies would allow better insight into the pertussis immune status in our country and hence the need for booster immunization.*

Keywords: Pertussis seroimmunity- acellular pertussis vaccine- pertussis resurgence.

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INTRODUCTION

Pertussis remains a serious infection in young infants. Most deaths occur in the first 3 months of life, before administration of the first dose of pertussis vaccine. Pertussis antibodies are transferred transplacental; but due to the lack of serologic correlates of protection, it is difficult to estimate the proportion of infants born with a protective levels of maternal antibodies.[1] Adolescents and adults have been identified as a source of transmission of pertussis to very young infants who are unimmunized or partially immunized and thus more vulnerable to disease morbidity and mortality[2]

Despite the widespread availability of 2 classes of effective vaccines, whole cell and acellular, pertussis has remained endemic and/or re-surged as a public health problem in many geographic locations. In 2015, the World Health Organization reported 142,512 pertussis cases globally, and estimated that there were 89,000 deaths. A recent CDC report estimates that there were 24.1 million pertussis cases and 160,700 deaths in children younger than 5 years in 2014 worldwide. While *Bordetella pertussis* circulates worldwide, disease rates are highest among young children in countries with low vaccination coverage rates, primarily in the developing world. In developed countries, the incidence of pertussis is highest among unvaccinated babies and rises again among teens.[3]

The international resurgence of pertussis, including the increase among the 7 to 10 year olds, is probably driven by a number of factors including a true return of the disease, increased public awareness, and better recognition and reporting by physicians, improved laboratory procedures such as PCR, and waning of immunity levels. It has also been hypothesized that genetic changes in the circulating strains of the organism may be a contributing factor through increasing vaccine failures. Another explanation might be the use of acellular component in the DTaP vaccines which is less potent than the old DTP vaccine. [4,5]

There is evidence that a true resurgence has occurred in 5 countries (Australia, Chile, Portugal,

USA and UK), four of which were exclusively using acellular pertussis (aP) vaccines. The observed increase in cases in Chile, which used whole cell pertussis (wP) vaccine, was considered to be related to factors other than the vaccine type, such as changes in surveillance and laboratory methods, and a recent decline in vaccination coverage.[6]

Among Egyptian children a positive trend in DPT immunization coverage was observed over the past years. It was significantly correlated with the reduction in the number of recorded cases, and since 2003 there were no recorded cases in Egypt as reported by the Ministry of Health and Population in 2005.[7] However, there are no recently published data on pertussis infection rates among the Egyptian population.

Waning of vaccine-induced immunity has been cited as one of the reasons for the observed epidemiologic trend. Naturally acquired immunity was reported to be persist for 7-20 years while vaccine-induced immunity remains valid for 4–12 years.[8]

Although pertussis vaccination is obligatory in Egypt, the recent emergence of whooping cough in other parts of the world stimulated us to investigate the current immune status against pertussis in in the pediatric age groups and in mother-neonate pairs. The ultimate objective is to trace any possible susceptibility of Egyptians to this devastating infectious disease and anticipate the need for further booster immunization.

METHODS

This was a cross-sectional study conducted on a stratified non-random sample. It was conducted in the period from July 2010 to April 2013. The study has been approved by the Local Ethics Committee of the Department of Pediatrics, Ain shams university. An informed consent was taken from the parents/caregivers before enrollment. All the subjects had received wP vaccine according to the Expanded Program of Immunization (EPI) adopted by the Egyptian Ministry of Public Health.

Study population:

The studied sample included 500 healthy subjects who were consecutively enrolled within the following groups

I. Mother-neonate pairs:

These were 75 healthy full-term newly born babies and their mothers enrolled consecutively from the Department of Gynecology and Obstetrics, Ain Shams University.

Inclusion criteria:

- Term pregnancy [37 weeks to 41 weeks according to the American college of obstetrics and gynecology definition].[9]
- Neonates with Apgar scores above 7 at one and five minutes

Exclusion criteria:

- Any maternal obstetric complication or chronic illness
- Intrauterine growth retardation
- Perinatal asphyxia

II. Infants group: it included 100 healthy infants whose ages ranged between 2 and 4 months.

III. Preschool children group: it included 90 children. Their ages ranged between 2 and 6 years.

IV. School-aged children group: it included 80 children whose ages ranged between 6 and 12 years.

V. Adolescent group: it included 80 adolescents whose ages ranged between 12 and 18 years.

The groups of infants, children and adolescents were recruited from the Casualty and Orthopedic Outpatient Clinic of the Children's Hospital, Ain Shams University and the Primary Health Care Unit in Obour City, Great Cairo Province.

Inclusion criteria:

- Healthy appearing subjects
- Normal growth patterns according to WHO standards

Exclusion criteria:

- Suspected primary or secondary immuno-deficiency
- Acute illness especially with fever and/or cough
- Any chronic illness.

Study Measurements:

I. Clinical evaluation:

For the ***mother-neonate pairs***; maternal clinical history was taken together with revising their obstetric medical records to exclude any chronic illness, intake of immunosuppressive medications or any acute obstetric illness or perinatal asphyxia. Assessment of the neonates was done using the APGAR score to ensure neonatal wellbeing. [10] The enrolled infants, children and adolescents were assessed by history taking and physical examination to exclude acute/chronic illness especially primary or secondary immunodeficiency.[11] WHO growth charts were used to assess growth.[12] History suggestive of previously acquired whooping cough was sought for.

II. Serum pertussis specific IgG was estimated by an enzyme linked immunosorbent assay (ELISA):

Two ml of venous blood were obtained by venipuncture from each participant and were collected into a gel vacutainer tube (Becton Dickinson, Oxford, UK). Blood was allowed to clot, and serum was separated by centrifugation (3500 rpm, 15 min, 25°C) and then stored in aliquots at -20°C until used for quantitative detection of Bordetella pertussis IgG by using ELISA kit *(Bordetella pertussis IgG ELISA. I Blinternational Gmbh. IBL@IBL-International. com, Hamburg, Germany).*The obtained OD (with a photometer at 450 nm) of the standards were plotted against their concentration on semi-logarithmic graph paper. The concentration of the samples could be read directly from the standard curve. According to the manufacture instruction, the concentrations were interpreted as followed: < 16 U/mL negative; 16 – 24 U/mL equivocal; > 24 U/mL positive

Statistical Analysis:

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) Version 21.0 (SPSS, Inc. Chicago III., USA) for Windows. Numerical data were summarized using means and standard deviations or medians and ranges as appropriate. Categorical data were summarized as percentages. Non normally distributed numeric variables were compared by Mann-Whitney test. For categorical variables, differences were analyzed with χ^2 (chi square) test and Fisher's exact test when appropriate. Differences among three groups were analyzed with analysis of variance between groups (ANOVA) and Bonferroni post hoc test. Correlations were determined by using Pearson's test or spearman Rho as appropriate. All p-values are two-sided. P-values < 0.05 were considered significant.

RESULTS

The demographic, clinical and laboratory data of the studied sample are displayed in table 1.

Positive pertussis IgG levels (>24 U/mL) were detected in 69 of the mothers (92%) and equivocal in the remaining six (8%), while positive pertussis IgG levels were present in 63 of the newborns (84%); 6 newborns (8%) had equivocal levels and the remaining 6 (8%) did not have any pertussis IgG antibody titer (figure 1). Fifty-five subjects in the infants' group (55 %) had positive pertussis IgG level; 7% had equivocal levels while the remaining 38 infants (38%) were negative for pertussis IgG titer. Seroimmunity to pertussis was detected in 82.2% of preschool children, 77.5% of school children and 75% in adolescents (figure 2).

The rates of pertussis IgG seroimmunity and concentrations were significantly higher among the mothers as compared to the neonates (table 2). Also, serum pertussis IgG titers among the neonates showed a significant positive correlation with the maternal titers (P=0.0001) (figure 3).

Mothers living in urban and suburban areas of Cairo had significantly higher serum pertussis IgG level than those living in rural areas such as the Sharkeya Governorate (Z=-2.83, p=0.005). Again, the children and adolescents living in urban and suburban areas had significantly higher frequency of seropositivity as compared to those living in rural areas (x2=6.1, p=0.04), but no significant difference was found in serum pertussis IgG concentrations between urban and rural residents (p>0.05).

Our results did not vary with gender (p > 0.05). On the other hand, serum pertussis IgG expression varied among the infants' group being higher in infants aged 12-24 months than those who were 6 months old by using the posthoc test, (p=0.005). Nevertheless, comparable levels of serum pertussis IgG were found between infants aged from 6-12 months and those aged less than 6 months. The preschoolers, school-aged children and adolescents showed comparable frequency of pertussis seroimmunity and IgG concentrations (p> 0.05). Also, in addition, there was no significant correlation between serum pertussis IgG titer and time elapsed after the last dose of pertussis vaccine whether in preschoolers, school-aged children or adolescents (p>0.05).

Table 1. Demographic and laboratory data of the studied infants, children and adolescents

	Infants n=100	Preschool children n=90	School-aged children n=80	Adolescents n=80
Age				
Mean (SD)	8.6 (5.7)	3.5±1	8.8±1.4	14.2±1.4
Range	2 – 24 months	2-5.5 yrs.	6.5-11 yrs.	12.5-17 yrs.
Gender n (%)				
Male	61 (61%)	47 (52.2%)	49 (61.3%)	33 (41.3%)
Female	39 (39%)	43 (47.8%)	31 (38.8%)	47 (58.8%)
Residence n (%)				
Urban and suburban	94 (94%)	68 (75.6%)	60 (75%)	63 (78.8%)
Rural	6 (6%)	22 (24.4%)	20 (25%)	17 (21.3%)
Weight (Kg)				
Mean (SD)	7.7 (2)	15.7±2.6	38.3± 7	43±8
Range	4 – 12	11-21	18-60	32-70
Height (Cm)				
Mean (SD)	68 (9)	94.3±9.2	125.2±9.2	149.8±7.8
Range	53 – 84	79-110	105-146	140-166
Serum pertussis IgG titre (U/ml)				
Mean (SD)	30 (42)	47.6±8.4	49.3±30.5	39.8±21.8
Range	2 – 170	5-125	10-120	8-100
Pertussis seropositivity				
Positive	55 (55%)	74(82.2%)	62(77.5%)	60(75%)
Equivocal	7 (7%)	2(2.2%)	9(11.3%)	5(6.3%)
Negative	38 (38%)	14(15.6%)	9(11.3%)	15(18.7%)
Duration since last dose of vaccine (years)				
Mean ± SD	-	3.05±1	8.3±1.4	13.6±1.4
Range	-	1.5-5	6-10.5	12-16.5

IgG: Immunoglobulin G; n: number; *p* value < 0.05 is significant; SD: standard deviation.

Table 2. Pertussis seroimmunity and serum pertussis IgG titres among the mother-neonate pairs

	Mothers	Neonates	Test	*p* value
Serum pertussis IgG titre (U/ml)				
Mean (SD)	95 (47)	56 (38)	t = -5.59	0.001
Range	20 - 170	5 – 170		
Pertussis seropositivity n (%)				
Positive	69 (92%)	63 (84%)	$X^2 = 6.2$	0.04
Equivocal	6 (8%)	6 (8%)		
Negative	0 (0%)	6 (8%)		

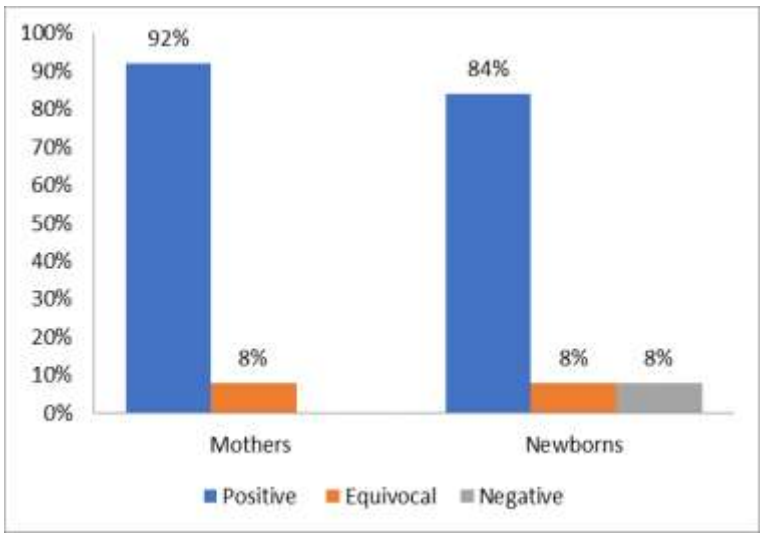

Figure 1. Frequency of pertussis IgG seropositivity in the mother-neonate pairs.

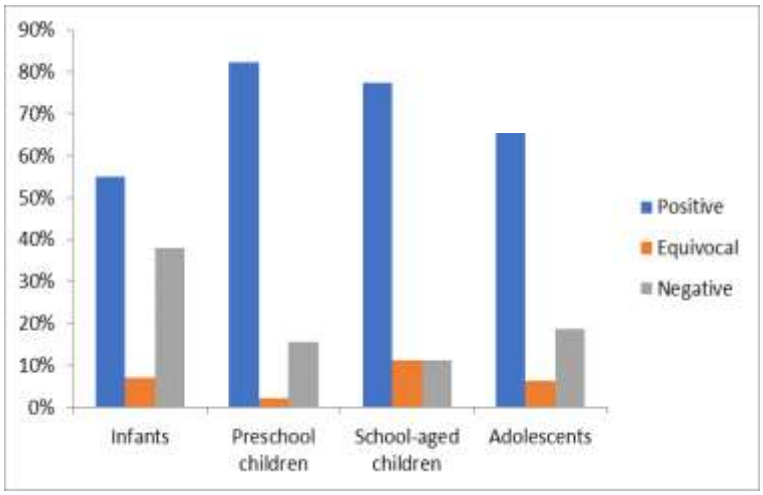

Figure 2. Variation of infants, children and adolescents according to the rates of pertussis seroimmunity

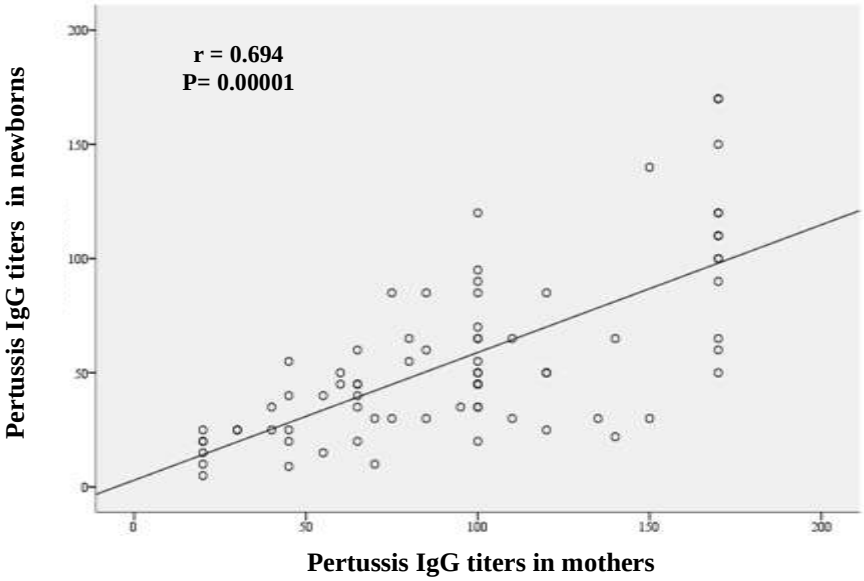

Figure 3. Positive correlation of pertussis IgG titers between mothers and newborns.

DISCUSSION

In the current study, all subjects received the wP vaccine which is implemented in the schedule of compulsory vaccination of Egyptian children at 2, 4, 6 and 18 months. A positive titre of serum pertussis IgG (>24U/mL) denoting seroimmunity was detected in 92% of the mothers, 84% of their newborns, 55% of the 2-24 months old infants, 82% of preschool children, 77.5% of school age children, and 75% of adolescents. The negative results among our sample may have resulted from failure of seroconversion after receiving the obligatory vaccine doses. Waning of immunity against pertussis could be an explanation for the negative results among older children and adolescents.

It had been reported that the efficacy of diphtheria-tetanus-whole-cell pertussis (DTwP) vaccine ranges from 46% to 92%.[13] Actually, the protective effect of the wP vaccine was reported to last for a varying period from 4-12 years in comparison to nearly 6 years of protection after acellular pertussis (aP) vaccine. [14,15] A study from Senegal, on children less than 15 years old, showed that three doses of a French wP vaccine had an overall efficacy of 55% against pertussis which is lower than our seroconversion rates.[16]

Low or undetectable pertussis-related antibody levels (maternal anti-pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin (PRN) IgG antibodies) were previously detected in parous women, suggesting increased susceptibility to infection. [17] Similarly, a previous study in Turkey reported that these anti-pertussis antibodies levels in cord and maternal blood in both preterm and term infant–mother pairs were generally inadequate to confer protection until the starting primary immunization. Transplacental anti-pertussis antibody transfer and antibody levels were reported to be inadequate in the cord blood of preterm infants, especially in those less than 32 weeks' gestation.[18] Several other studies reported low levels of protective pertussis antibodies in females during the child-bearing period.[19-21]

This was not the case in our series where the high frequency of maternal seroimmunity against pertussis and the adequate serum pertussis IgG concentrations reflected a better immune status which is possibly achieved by the whole-cell vaccine usage and might be further enhanced by exposure to subclinical infection.

The frequency of pertussis IgG seroimmunity and serum pertussis IgG concentrations were significantly higher among mothers as compared to the neonates in the current study. This is expected because transplacental antibody transfer may not be sufficient to confer the same rates of seroimmunity in the offspring. A previous study found that even with efficient transplacental passage of pertussis antibodies, neonates have limited measurable protection as detected by cord blood sampling.[17] However, the serum pertussis IgG titers of our group of neonates correlated positively to those of the mothers denoting that the higher the level of maternal seroimmunity, the higher the chance that her fetus acquires antibodies. The same conclusion was noted in previous studies.[17, 22]

Among our series, subjects living in rural areas seemed to have less protective levels of pertussis IgG seroimmunity than their peers living in urban and suburban areas. This could be related to the degree of commitment of parents to the vaccination schedule in rural areas where lower parental education levels are expected. The efficacy of vaccine preservation and delivery might also differ according to residential classification.

Gender did not influence the seroimmunity in our study; the same was reported by several other investigators.[23-26]

In the current study, serum pertussis IgG titres increased with age among the infants' group being lowest below 6 months of age and higher in the 12 to 24 months old which is expected due to the receival of booster doses of the vaccine. A relevant study showed that pertussis antibody levels >30 U/ml were observed with higher frequency in infants younger than 2 years of age who completed the vaccination schedule . [27] Another study revealed that vaccinated 1-2-year-old infants and 17-18-year-old adolescents had the highest serum pertussis IgG titers. [24]

An interesting observation in a group of Turkish children was that pertussis antibodies increased after the age of 6 years and a further increase was observed between 7 and 12 years. Since there is no booster against pertussis at this age, this increase probably reflects the acquisition of natural immunity following the beginning of elementary school, where children join a new crowded community with higher rates of exposure to communicable diseases.[25]

On the other hand, pertussis seroimmunity, among our series, did not vary significantly between preschoolers, school-aged children and adolescents. This finding needs to be further validated in wider scale studies although waning of immunity would be expected beyond these age groups. The mean duration since the last dose of pertussis vaccine was 3, 8, and 13.5 years in the preschoolers, school-aged children, and adolescents

respectively. It has been reported that seroimmunity following pertussis vaccination offers a high degree of protection for 3 years after completion of infant immunization, but the vaccine antibodies begin to decrease 3-4 years after the last dose.[28] Other investigational studies noted that pertussis vaccine antibodies start to wane after 4-12 years [8] or 6-9 years.[29]

A previous study reported that adolescents and older children are often the most affected by an outbreak of pertussis. Many adults may also become infected but may not develop significant symptoms. Vaccine-induced immunity seems to wane from childhood to adolescence but does not appear to wane further into adulthood. These findings suggest that adding an additional booster dose of acellular pertussis vaccine for adolescents may be helpful in further reducing the development of clinical disease in the community.[30]

This pilot study has some limitations indeed. The relatively small sample size is inadequate to augment conclusions in a large population such as the Egyptian. Also, the uneven distribution of the sample according to residency hindered reaching a solid conclusion about the seroimmunity among rural residents.

In conclusion, the subjects enrolled in the current study have relatively good pertussis seroimmunity rates and antibody titres that could be due to the use of whole cell vaccine in the national EPI schedule during the study period and possibly augmented by subclinical exposure to natural infection. The failure rates detected could be due to inefficient vaccination in the younger groups or waning of immunity in the older children and adolescents. The lower seroimmunity observed among rural residents might reflect a difference in vaccination efficiency or may be a false impression due to the smaller proportion of this sector among the studied sample. Wider scale studies would allow better insight into the Egyptian immune status against and hence the possible need for booster immunization. Also, investigating the cause of lower pertussis seroimmunity in the countryside is warranted.

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